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# SISTER CHROMATID EXCHANGES IN MAMMALIAN MEIOTIC CHROMOSOMES

by

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16. ABSTRACT

Very little is known about sister chromatid exchanges (SCEs) in meiotic cells--only that they occur (1) and reveal frequency and distribution patterns apparently unaffected by cross-over (CO) exchange conditions in those cells; (2) unfortunately, the number of studies from which to draw these general conclusions is small due a variety of methodological difficulties. Only a few organisms have been analyzed for baseline SCEs in meiotic tissue. No information pertaining to the extent or significance of SCE induction by mutagens during meiosis is available. In the present work, an Armenian hamster (Cricetulus migratorius) spermatocyte system is described in terms of its methodological features and its applications for baseline SCE analyses. This system appears to have considerable promise for comparative studies of mechanisms involved in SCE and CO, and for development as an assay to detect and determine the significance of induced SCEs in meiotic cells.

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INTRODUCTION

Very little is known about sister chromatid exchanges (SCEs) in meiotic cells -- only that they occur (1) and reveal frequency and distribution patterns apparently unaffected by cross-over (CO) exchange conditions in those cells (2). Unfortunately, the number of studies from which to draw these general conclusions is small due a variety of methodological difficulties. Only a few organisms have been analyzed for baseline SCEs in meiotic tissue. No information pertaining to the extent or significance of SCE induction by mutagens during meiosis is available.

Interest in SCEs first arose in studies concerned with meiosis. The occurrence and frequency of SCEs in meiocytes were addressed in early cytological work with Drosophila and maize (3,4). SCEs have been likened to meiotic COs between homologues in that both exchanges are forms of chromatid recombination. Although these exchange-types are fundamentally different in many ways (i.e., in their cell-cycle times of occurrence, frequencies and distributions, inducibilities and apparent genetic significance), their concurrence in the same chromosomes is unique and poses special analytical problems. Strictly morphological assessments have relied upon chiasmata to signal COs (5), and have been of very limited use for detecting SCEs. Opportunities to resolve SCEs and COs directly came with the autoradiographic methodology of Taylor and colleagues (6,7). Grasshopper spermatocytes were characterized for patterns of semi-conservative replication and crossing-over and incidentally for SCE as a confounding factor leading to deviations in expected label distribution patterns (7,8). SCE frequencies in meiotic chromosomes were reported in subsequent autoradiographic studies

of insects and plants (see ref. 2). One such study in grasshoppers provided evidence that the formation of these SCEs was not restricted to the penultimate (mitotic) DNA synthesis period prior to their detection at meiosis (1). A binucleate spermatocyte revealed a specific chromosomal SCE in only one nucleus rather than in both of the nuclei, thus indicating that it occurred during the ultimate (meiotic) DNA synthesis period.

Ten years ago, Latt developed in vitro 5-bromodeoxyuridine (BrdU) - fluorescent dye methodology for high resolution analyses of SCEs, chromosome structure, and replication kinetics in mammalian cells (9,10). With the significant improvement of permanent staining [i.e. the fluorescent-plus-Giemsa (FPG) step added by Perry and Wolff (11)] BrdU-dye methodology became widely used for studies of SCE induction by mutagens (12,13). From toxicological as well as purely genetic standpoints, the possible mechanistic and interactive relationships of SCEs with meiotic COs (homologous non-sister chromatid exchanges) were of interest. However, meiotic cells which earlier comprised a focus for SCE analyses were among the least favorable dividing tissues for BrdU-differential staining assessments. Germ cells are difficult to grow in culture and maintain through differentiative stages of meiosis. Although in vivo trials seemed promising, initial attempts to effect differential staining in meiotic cells of locusts (14) and rodents failed—apparently because of rather exacting requirements in regard to BrdU dose to dividing cells. Excessive analogue availability to replicating germ cell DNA results

in cytotoxicity (reduced numbers of meiotic metaphase cells) whereas too little does not accomplish the levels of labelling necessary for clear differential staining and elucidation of SCEs. The unusual sensitivities of germ cells may be related to their unusually long DNA synthesis and cell-cycling times [i.e. approx. 14h and 30h respectively in mouse late spermatogonial cells (15)].

Eventually, suitable BrdU-dye methods for studying SCEs and COs in insects and rodents were developed. Although only a few studies with these systems have been conducted, several reviews concerned with the implementation of the new techniques and with the findings to date have been reported (16 -18). Systems utilizing intact mammals such as mice or hamsters would be useful due to their particular relevance for genotoxicity studies; however, such studies have proven relatively difficult to perform technically. Cytotoxicity has continued to be a problem in at least some of the systems, and SCE analyses have been restricted to certain bivalents. In the present work, an Armenian hamster (Cricetulus migratorius) spermatocyte system is described in terms of its methodological features and its applications for baseline SCE analyses. Initial procedures and applications for characterization of COs have been reported elsewhere (19). This system appears to have considerable promise for comparative studies of mechanisms involved in SCE and CO, and for development as an assay to detect and determine the significance of induced SCEs in meiotic cells.

METHODOLOGICAL DEVELOPMENT

Historical

Autoradiography. Taylor and associates determined that radiolabelled DNA is semi-conservatively distributed in germ cells (7). Grasshopper spermatocyte chromosomes which incorporated $^{3}$H-thymidine two cell-cycles earlier during spermatogonial DNA synthesis revealed differentially labelled sister chromatids at metaphase. As progression from primary to secondary spermatocytes involves reduction division to the haploid state without further DNA synthesis, identical chromosome labelling characterizes the two cell types. Autoradiographic analyses revealed label switches between sister chromatids (SCEs) and between non-sister chromatids of homologous chromosomes (COs).

As noted by Taylor, random chromatid involvement in crossing-over dictates that half of the recombinants should be between like-labelled non-sisters and therein be "hidden" from detection by altered label patterns. The other half of CO recombinants should involve unlike-labelled chromatids, and lead to "visible" disruptions to the homologous chromosomal labelling patterns. Hidden COs would be detectable only by the presence of a chiasma whereas visible COs would be indicated by corresponding isolabelling in the homologues, as well as by chismata. However, analyses of these CO exchanges and their distinctions from SCEs is less than straightforward for several reasons. Exchanges in the synapsed regions of primary meiocyte bivalents are difficult to resolve and confusing to interpret with the limited resolution

of autoradiography. Thus, assessments have frequently been carried out in secondary meiocyte (metaphase II) cells. Yet, these cells are more removed from the cross-over event and chiasmata are no longer present to signal COs. As discussed below, SCEs and COs can mimic one another's patterns in metaphase II chromosomes and pose significant problems to their individual identification and analysis.

BrdU-differential staining. BrdU-differential staining techniques follow the same principles of label kinetics and exchange in meiosis as those forming the basis of autoradiographic methods. Meiotic sister chromatids are differentiated through asymmetry in substitution of BrdU for thymidine and staining with a BrdU-sensitive dye. Exchange points then appear as abrupt discontinuities in chromatid staining patterns. In recent BrdU-dye studies of locust meiotic chromosomes, a considerable amount of information bearing on SCEs, as well as classical concepts of crossing-over, was reported (2,17). Meiotic SCEs were concluded to be similar in nature to to mitotic SCEs. Comparable baseline levels are encountered and both reveal distributions which appear to be random and frequencies which depend upon chromosomal size.

A particular obstacle in carrying out in vivo studies in mammals with the BrdU-dye technique involved the rapidity of liver degradation of BrdU (20) which effectively prevented adequate levels of analogue from being incorporated into DNA. This problem was overcome by various means of continually replenishing BrdU to the animal. The first spermatogonial and meiotic cell SCE studies

in mammals were carried out in male mice given a series of injections of BrdU (21). In the latter cell-type, unsatisfactory chromatid differentiation in all but the late-replicating sex-bivalent greatly restricted analyses. Extensive BrdU incorporation into earlier replicating autosomes appeared to be somewhat cytotoxic. Subsequently, more successful mammalian meiotic cell studies of cross-overs and SCEs have been reported in male (22) and female (23) mice, and in Armenian hamsters (19). BrdU labelling techniques employed in these studies involved multiple injections, infusion, or slow release of analogue from internal charcoal-BrdU deposition or implanted BrdU pellets. Mouse oocytes were labelled *in vitro* as part of a novel approach developed by Polani and colleagues (23). Embryonic ovaries were incorporated with BrdU under culture conditions and then transplanted to adults for *in vivo* maturation.

The first staining evidence of COs in a mammal was obtained in Armenian hamster spermatocytes (24). A subsequent study (19) provided more detail and a characterization of replication kinetics and SCEs in the meiotic cells as well. This animal was originally selected for meiosis studies because it was known to have a clear chiasma in the sex bivalent (25,26). Earlier mouse studies had revealed superior differential staining results in the sex bivalent where unfortunately COs were not discernible (21). Recent technical modifications have significantly lessened cytotoxicity problems and improved chromosome labelling results in this system. General methodology and initial results from SCE analyses using Armenian hamsters are described below.

General Methods: Armenian Hamsters

An overview of *in vivo* methodology for analyzing SCEs and COs in Armenian hamsters is given in Fig. 1. Young (3-5 mos.) male hamster (approx. 35g) germ cells are labelled using BrdU pellet methodology (24,27). Fifty mg pure BrdU pellets are pressed, coated with paraffin over most of the surface area (27) and implanted into lightly anesthetized (Metofane) animals. After 2 1/2 days, the paraffin and any remaining BrdU are removed. Second division spermatogonial metaphases may be harvested at this time (after a 4h colchicine treatment) or harvests may be delayed until days 16-18, at which time second division (post-labelling) spermatocyte diplotene through metaphase II cells are obtainable. Standard cytogenetic harvest, slide preparation, and fluorescent or fluorescent-plus-Giemsa differential staining procedures (13) may be used for analyzing exchanges in meiotic chromosomes.

It is notable that the paraffin-coating step in the preparation of BrdU pellets has led to significantly improved results as compared with those obtained using injections and uncoated pellet methods. It would appear that the slower, steadier release of analogue attained after coating the pellet reduces the heavy pulse effect which is likely to be harsh on these cells. As a result, fully labelled primary and secondary spermatocytes (Fig. 2) are common, rather than only occassionally obtainable.

## HAMSTER GERM CELL ANALYSES

### SCEs In Primary Spermatocytes

It is difficult to assess SCEs in all chromosomes of primary spermatocytes due to the rarity of such cells with adequate morphological clarity in all chromosomal bivalents. However, the monochiasmate sex bivalent and several of the larger autosomal bivalents (Fig. 3) which appear as mono- or multi-chiasmate are relatively easy to analyze (SCE frequencies per bivalent are noted below). The sex bivalent has also been particularly well studied for hidden and visible types of COs (19), and for simultaneous CO and SCE staining patterns (Fig. 4). On either side of the chiasma, X and Y short arms reveal chromatid differential staining (i.e. Fig. 4a) or isolabelling for bright or dull fluorescence (i.e. Fig. 4b). These hidden and visible COs between the sex chromosomes have been noted to occur in approximately 1:1 ratios in young Armenian hamsters (19) which agrees with similar CO-type ratios reported in locusts (28) and mice (16).

A variety of staining patterns reflecting combinations of SCEs and crossovers are evident in the sex bivalents of Fig. 4c-g. For example, in Fig. 4e an SCE is apparent between the synapsed short arms in the chiasma. This observation is in keeping with the concept that pre-CO sister chromatids manifest a pairing attraction across the chiasma (5). Another exchange in this bivalent is indicated by the mismatching of staining diagonally

across the chiasma (or between long and short arms in two of the apposing chromatids). This pattern has been noted in locusts (28), mice (22) and hamsters (19), and it is uncertain as to whether it should be attributed to an SCE or to additional crossing-over (28).

One additional source of confusion stemming from SCEs is the production of isolabelling similar to that resulting from visible COs where, in fact, no COs occurred. This problem was addressed by Moens in an early tritium - labelling study (29). The bivalent examples in Fig. 4 f. and g. are at the third division and reveal isolabelled or uniformly stained regions in the Y chromosome long arms. These patterns were likely generated by SCEs in the first and/or second of three replication periods. Since the sex chromosomes are generally quite late replicating, it is not uncommon to find cells in which X and Y chromosomes have replicated three times, and most autosomes only twice, after the start of BrdU labelling.

SCEs In Secondary Spermatocytes

Meiotic metaphase II chromosomes are morphologically easy to analyze; however, potential misinterpretations of differential staining patterns present a unique problem in this cell type. Since chiasmata have disappeared, it becomes impossible to distinguish between hidden crossing-over and failure to cross-over. Further, mimicry between CO and SCE staining patterns in secondary spermatocytes prevents any precise determinations of SCE frequencies and distributions. Jones and co-workers (2, 30-32) have thoroughly addressed this problem in studies with grasshoppers and locusts, and have developed means by which to indirectly assess SCE levels in such cells (see below).

Fig. 5 illustrates how typical SCE and CO stain images can be misleading in instances where they may not be what they appear to be. Selected hamster metaphase II chromosomes with accompanying theoretical models of recombination show how apparent switches between differentially stained sister chromatids might actually have arisen from CO events only, and how SCEs might be obscured by COs and therefore overlooked. Clearly, SCEs might be under- or over-estimated in attempts to directly determine their frequencies in secondary spermatocyte cells.

Replication Kinetics In Spermatogonia

A final source of confusion in differential staining analyses of cross-overs and SCEs in meiocytes is that which can arise from significant intra-chromosomal variations in the timing of DNA replication. The uneven labelling of spermatogonial DNA with BrdU can result in an appearance of isolabelling (resembling visible cross-overs) in meiotic metaphase I and metaphase II cells. Fig. 6 reveals several Armenian hamster chromosomes which should be considered in this light. When boundaries of differentially replicating segments occur at or near frequent cross-over sites, as is the case in the nos. 3 and 6 chromosomes, it is difficult to assess the derivation of isolabelling detected at meiosis. By contrast, isolabelling due to differential replication in the X long arms is not problematic in its interpretation because crossing-over does not occur in that region (19). While misinterpretations of isolated patterns have a direct effect on CO analyses, they can also indirectly affect SCE analyses in secondary spermatocytes (see below).

SCE Frequencies

SCE frequencies in Armenian hamster meiotic chromosomes can be determined by direct analysis of selected primary spermatocyte bivalents, and by indirect analysis of secondary spermatocyte chromosomal complements. As noted above the larger chromosomes no. 1, no. 6, and X-Y bivalents are especially amenable to SCE analysis. The chromosomes may also be identified in spermatogonial cells on the basis of size and centromere positions (25). Table 1 depicts comparative analyses of SCE frequencies in these chromosome pairs observed in spermatogonial and primary spermatocyte cells. No significant difference in SCE/chromosome levels between the two cell types were found. It is likely that the slightly lower values observed in the sex-bivalent (similar in size to the autosomes) may be due to the differential replication kinetics in the X chromosome long arms (Fig. 6), which can obscure the uniformity of clear chromatid differentiation along the entire length of the chromosome.

Although SCEs are not always reliably identifiable in secondary spermatocyte chromosomes, frequencies may be deduced after accounting for the proportion of detectable exchange sites attributable to COs. Preliminary results following the basic approach developed by Tease and Jones (2) for analyzing SCEs in secondary spermatocyte cells are presented in Table 2. In four individual hamsters, the mean chiasma frequencies were determined in primary spermatocytes (diakinesis) and the total number of exchange boundaries (reciprocal and non-reciprocal) per cell were recorded for

secondary spermatocytes. The expected number of detectable exchange boundaries due to visible crossing-over would equal one-half of the chiasma frequencies; thus, the exchanges in excess of this number are interpreted as arising from SCEs (two excess exchanges/SCE). Values calculated for haploid secondary spermatocytes are doubled to obtain SCE frequencies for diploid cells. Average frequencies determined in this manner for the different animals ranged from 0.2 to 1.4 SCEs/ meiotic cell. These frequencies are approximately those which would be predicted from the individual bivalent analyses (Table 1), taking into account that the bivalents analyzed were among the largest of the chromosomal complement. They also closely match the average spermatogonial cell frequencies of $1.4 \pm 0.8$ and $1.7 \pm 0.9$ observed in two separate hamsters (20 cells each).

It is notable that the germ cell SCE frequencies obtained for hamsters in this study are consistent with those reported by BrdU-labelled mice and locusts despite some differences in labelling techniques. Values in mice of approximately $\leq 2$ SCEs/spermatocyte bivalent (22), and 0.10 SCEs/oocyte bivalent (16) have been determined. In locusts (2), values of 0.14 to 0.37 were obtained for different chromosomes in primary spermatocytes. Slightly higher frequencies of 0.28 and 0.89 (per bivalent) were calculated in anaphase I and metaphase II cells for the same chromosomes, respectively. The mammalian meiotic SCE frequencies also tend to be a little lower than those reported for other insects and plants studied with autoradiography (see Fig. 2). This is not surprising in view of the technical/procedural differences in DNA labelling.

As a final point, it remains uncertain as to whether there is a technical or biological explanation for why rodents generally reveal lower SCE frequencies in their germ cells as compared with somatic cells (18) while insects appear to show similar frequencies in somatic and germ tissues (17).

SUMMARY

Meiotic cells have been, and remain, a relatively difficult tissue in which to study SCEs. Experimental evaluations have had to contend with various problems unique to germ cells, i.e. poor in vitro growth, unusual cytotoxicities (from BrdU), trying chromosome morphologies, and confusion between chromosome label patterns generated by SCEs, COs, and replication kinetics. Nevertheless, BrdU differential staining techniques in insects and rodents have progressed to the point where SCE frequencies can be reliably determined.

In the described Armenian hamster system, SCEs may be directly resolved in selected primary spermatocyte bivalents and indirectly assessed in secondary spermatocyte cells. SCE frequencies determined from spermatogonial, primary spermatocyte, and secondary spermatocyte cells are in good agreement. The system should be applicable for studies of possible mechanistic similarities between SCE and CO exchange, and for evaluating genotoxic effects. Experimental evidence in Drosphila (33) argues against a very close molecular relationship between these forms of breakage and recombination. However, it is likely that new information can be gained from cells in which normal SCE and CO frequencies and distributions can be characterized, and from studies of the consequences to these events after various mutagenic perturbations to pre-meiotic and meiotic DNA.

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FIGURE LEGENDS

Figure 1 - Diagrammatic representation of in vivo protocol for analyzing chromatid exchanges in hamster meiotic chromosomes. BrdU exposure over 2 1/2 days effects analogue labelling over the penultimate and ultimate germ cell DNA synthesis periods. BrdU substitution (broken lines) in sister chromatids is symmetrically unifilar after one round of replication, and unifilar vs bifilar after two rounds of replication. The latter asymmetry of labelling characterizes diplotene through metaphase II chromosomes harvested on days 16-18. Staining of these chromosomes with 33258 Hoechst fluorescent dye results in dull fluorescence (black shading) over the more heavily-labelled chromatid and relatively brighter fluorescence (grey shading) over the less heavily labelled chromatid. Various florescent-plus-Giemsa procedures are also effective. Differential staining of sister chromatids enables the detection of SCEs, as well as cross-overs between unlike stained non-sister chromatids.

Figure 2 - Representative primary spermatocyte a and secondary spermatocyte b revealing chromatid differential staining in most or all chromosomes. Each chromosome is individually identifiable on the basis of morphology and cross-over recombination criteria (9,5).

Figure 3 - SCEs evident in the number 6 autosomal bivalent which, in this instance, appears monochiasmate. Two SCEs are indicated (→).

Figure 4 - Various chromatid exchange staining patterns in monochiasmatic sex-bivalents. Bivalent a exemplifies hidden crossing-over. Recombined short-arm segments have not altered the continuity of staining into the chiasma. Like-stained chromatids are in diagonal apposition across the chiasma, which is consistent with pairing attraction between regions previously in sister orientation. Bivalent b shows visible crossing-over in which a bright short-arm segment was exchanged with a dull short-arm segment to result in isolabelling for bright or dull on either side of the chiasma. Bivalents c-e reveal hidden or visible cross-overs, and an additional exchange (SCE?, CO?) in the cross-over region as evidenced by the mismatched staining diagonally across the chiasma. Bivalent e also reveals an exchange between the paired X-Y short arms ( → ), which is interpreted as a pre cross-over SCE, and thus is further evidence for sister chromatid pairing attraction in the chiasma. Bivalents f and g reveal dull isolabelling (long arms) consistent with that expected from visible crossing over. However, chiasmata involving long arms of the sex chromosomes have never been observed (homology appears restricted to short arms). It is presumed that the mimicry of cross-over staining patterns observed here actually arose from SCEs occurring in pre-final replication periods of these third division bivalents.

Figure 5 - Mimicry of staining appearances by SCEs and cross-overs in representative secondary spermatocyte chromosomes (no. 1). In a, a cytological example revealing the typical appearance of an SCE is depicted

with a possible recombination mechanism involving only cross-overs (proximal hidden, distal visible). Similarly, in b, a cytological example revealing the typical appearance of a visible cross-over (dull isolabelling) might actually have been derived via the accompanying mechanism involving a proximal hidden cross-over and a distal SCE. In c, a more complicated staining pattern involving the "appearance" of cross-over and SCE images is followed by examples of two possible mechanisms involving either two cross-overs and one SCE, or one cross-over and two SCEs.

Figure 6 - Replication kinetics in Armenian hamster spermatogonial chromosomes partially labelled (early mid-S) with BrdU and stained at the next metaphase with 33258 Hoechst dye. Examples of chromosome numbers 3 and 6, a, and the X chromosome, b, (from ref. 19) reveal intra-chromosomal regions with markedly different times of replication, and uneven labelling with BrdU, to result in different staining patterns ( → ). When these regions are large or pronounced in meiotic chromosomes, they may be confused with isolabelling resulting from visibletype cross-overs.

TABLE 1 - SCE Frequencies in Specific Chromosome-types Analyzed at Spermatogonial and Primary Spermatocyte Metaphase Stages

| Chromosome Pair | Spermatogonia[a] | 1° Spermatocytes[b] (Diakinesis-Metaphase I) |
|---|---|---|
| No. 1 | 0.38 | 0.32 |
| No. 6 | 0.37 | 0.36 |
| X-Y | 0.28 | 0.23 |

[a] SCE frequencies calculated from 40 cells; 2 young (3-5 mos.) Armenian hamsters

[b] SCE frequencies calculated from 25 cells for each bivalent type; 5 young (3-5 mos.) Armenian hamsters

TABLE 2 - Meiotic Cell SCE Frequencies Indirectly Assessed in Secondary Spermatocytes from Armenian Hamsters

| Armenian Hamster (4-6 mos.) | No. of 2° Spermatocytes | Observed Exchange[a] Boundaries/Cell | Chiasma Frequencies[b] In 1° Spermatocytes | Expected Visible[c] Cross-Overs/Cell | SCE Frequencies[d] (2° Spermatocytes) | SCE Frequencies[e] (1° Spermatocytes) |
|---|---|---|---|---|---|---|
| A | 23 | 9.7 | 17.4 | 8.7 | 0.5 | 1.0 |
| B | 19 | 10.0 | 17.2 | 8.6 | 0.7 | 1.4 |
| C | 14 | 10.0 | 17.4 | 8.7 | 0.7 | 1.4 |
| D | 36 | 8.8 | 17.3 | 8.7 | 0.1 | 0.2 |

a Mean number of stain-contrast junctions (reciprocal + non-reciprocal) per cell

b Mean number of chiasmata calculated from 30 1° spermatocytes (diplotene-diakinesis) for each animal

c Visible cross-over frequencies = 0.5 x chiasma frequencies

d SCE frequencies = 1/2 (since 2 exchange boundaries are observed/SCE) of (observed exchange boundaries - expected visible cross-overs)

e Double the SCE frequencies calculated for haploid secondary spermatocytes

REFERENCES:

1. John, B., and K.R. Lewis (1965). The meiotic system. In Protoplasmatalogia VI/F/I., Springer-Verlag, Wien, pp.

2. Tease, C., and G.H. Jones (1979). Analysis of exchanges in differentially stained meiotic chromosomes of Locusta migratoria after BrdU-substitution and FPG staining. II. Sister chromatid exchanges. Chromosoma 73:75-84.

3. Beadle, G.W., and S. Emerson (1935) Further studies of crossing-over in attached-X chromosomes of Drosophila melanogaster. Genetics 20:192-206.

4. Schwartz, D. (1953). Evidence for sister-strand crossing over in maize. Genetics 38:251-260.

5. Darlington, C.D. (1937). Meiosis in diploids and polyploids. In Recent Advances In Cytology. The Blakiston Company, Philadelphia, pp. 85-133.

6. Taylor, J. H., Woods, P.S., and W.L. Hughes (1957). The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. Natl. Acad. Sci. 43:122-128.

7. Taylor, J.H. (1965). Distribution of tritium-labeled DNA among chromosomes during meiosis. J. Cell Biol. 25:57-67.

8. Taylor, J.H. (1983). Keynote address of this Symposium.

9. Latt, S.A. (1973). Microfluorometric detection of DNA replication in human metaphase chromosomes. Proc. Natl. Acad. Sci. 70:3395-3399.

10. Latt, S.A. (1974). Sister chromatid exchanges, indices of human chromosome damage and repair: detection by fluorescence and induction by mitomycin C. Proc. Natl. Acad. Sci. 71:3162-3166.

11. Perry, P. and S. Wolff (1974). New Giemsa method for the differential staining of sister chromatids. Nature 251:156-158.

12. Perry, P.E. (1980). Chemical mutagens and sister chromatid exchange. In Chemical Mutagens-Principles and Methods for Their Detection, Vol. 6, J. de Serres and A. Hollaender, ed., Plenum Press, New York, pp. 1- 39.

13. Latt, S.A., Allen, J., Bloom, S.E., Carrano, A., Falke, E., Kram, D., Schneider, E., Schreck, R., Tice, R., Whitfield, B., and S. Wolff (1981). Sister chromatid exchanges: a report of the gene-tox program. Mutat. Res., 87:17-62.

14. Jones, G.H. (1977). A test for early terminalization of chiasmata in diplotene spermatocytes of Schistocerca gregaria. Chromosoma 63:287-294.

15. Monesi, V. (1962). Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine. J. Cell Biol. 14:1-18.

16. Polani, P.E., Crolla, J.A., and M.J. Seller (1981). An experimental approach to female mammalian meiosis: differential chromosome labeling and an analysis of chiasmata in the female mouse. In Bioregulators of Reproduction, Vogel and Jagiello, eds., Acad. Press, New York, pp.

17. Jones, G.G., and C. Tease (1981). Meiotic exchange and analysis by molecular labelling. In Chromosomes Today , Vol. 7, M.D. Bennett, M. Bobrow, and G. Hewitt, eds., George Allen and Unwin LTD, London, pp. 114-125.

18. Allen, J.W. (1982). SCE and meiotic crossover exchange in germ cells. In Sister Chromatid Exchange (Progress and Topics in Cytogenetics, Vol. 2). A.A. Sandberg, ed., Alan R. Liss, Inc., New York, pp. 297-311.

19. Allen, J.W. (1979). BrdU-dye characterization of late replication and meiotic recombination in Armenian hamster germ cells. Chromosoma 74: 189-207.

20. Kriss, J.P., Maruyama, Y., Tung, L.A., Bond, S.B.; and L. Revesz (1963). The fate of 5-bromodeoxyuridine, 5-bromodeoxycytidine, and 5-iododeoxycytidine in man. Cancer Res. 23:260-268.

21. Allen, J.W. and S.A. Latt (1976). In vivo BrdU-33258 Hoechst analysis of DNA replication kinetics and sister chromatid exchange formation in mouse somatic and meiotic cells. Chromosoma 58, 325-340.

22. Kanda, N., and H. Kato (1980). Analysis of crossing over in mouse meiotic cells by BrdU labelling technique. Chromosoma 78:113-121.

23. Polani, P.E., Crolla, J.A., Seller, M.J. and F. Moir (1979). Meiotic crossing over exchange in the female mouse visualized by BudR substitution. Nature 278:348-349.

24. Allen, J.W., Shuler, C.F. and S.A. Latt (1978). Bromodeoxyuridine tablet methodology for in vivo studies of DNA synthesis. Somatic Cell Genet. 4:393-405.

25. Lavappa, K.S. and G. Yerganian (1970). Spermatogonial and meiotic chromosomes of the Armenian hamster, Cricetulus migratorius. Exp. Cell Res. 61:159-172.

26. Solari, A.J. (1974). The relationship between chromosomes and axes in the chiasmatic XY pair of the Armenian hamster (Cricetulus migratorius) Chromosoma 48:89-106.

27. McFee, A., Lowe, K.W. and San Sebastian, Jr. (1983). Improved sister chromatid differentiation using paraffin-coated bromodeoxyuridine tablets in mice. Mutation Res. Letters. 119/1:83-88.

28. Tease, C. and G.H. Jones (1978). Analysis of exchanges in differentially stained meiotic chromosomes of Locusta migratoria after BrdU-substitution and FPG staining. I. Crossover exchanges in monochiasmate bivalents. Chromosoma 69:163-178.

29. Moens, P.B. (1966). Segregation of tritium-labeled DNA at meiosis in Chorthippus. Chromosoma 19:277-285.

30. Jones, G.H. and T. Craig-Cameron (1969). Analysis of meiotic exchange by tritium autoradiography. Nature 223:946-947.

31. Craig-Camero T.A. and G.G. Jones (1969). The analysis of exchanges in tritium-labelled meiotic chromosomes I. Schistocerca gregaria. Heredity 25:223-232.

32. Jones, G.H. (1971). The analysis of exchanges in tritium-labelled meiotic chromosomes. II Stethophyma grossum. Chromosoma 34:367-382.

33. Gatti, M., Pimpinnelli, S., and B.S. Baker (1980). Relationships among chromatid interchanges, sister chromatid exchanges, and meiotic recombination in Drosophila melanogaster. Proc. Natl. Acad. Sci. 77:1575-1579.

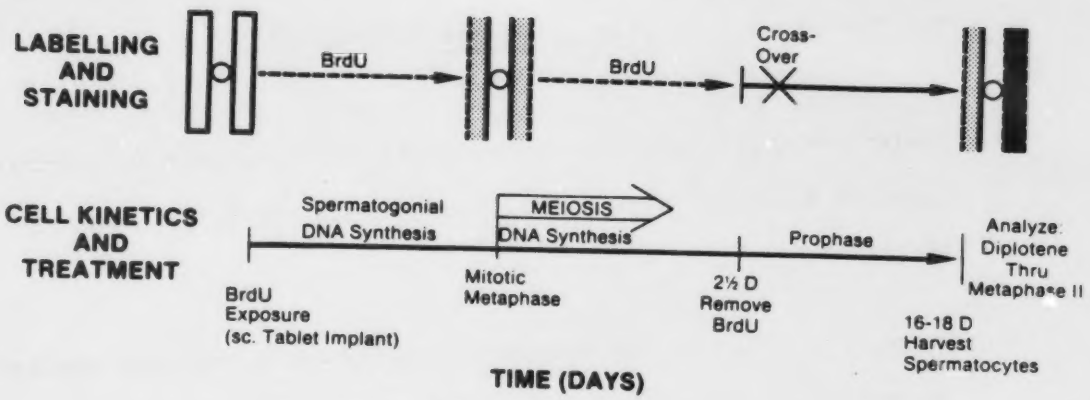


figure 1

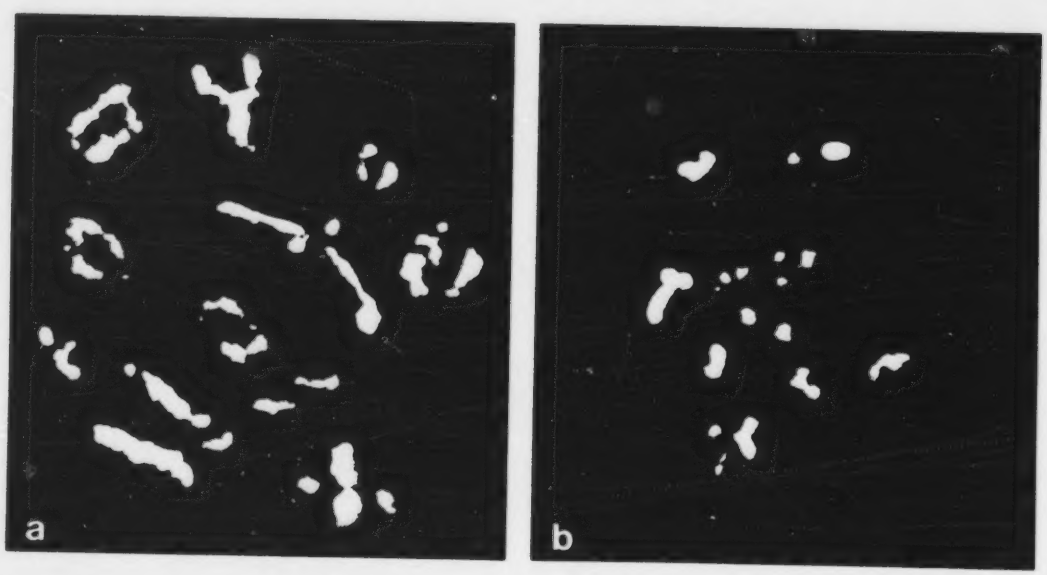


figure 2

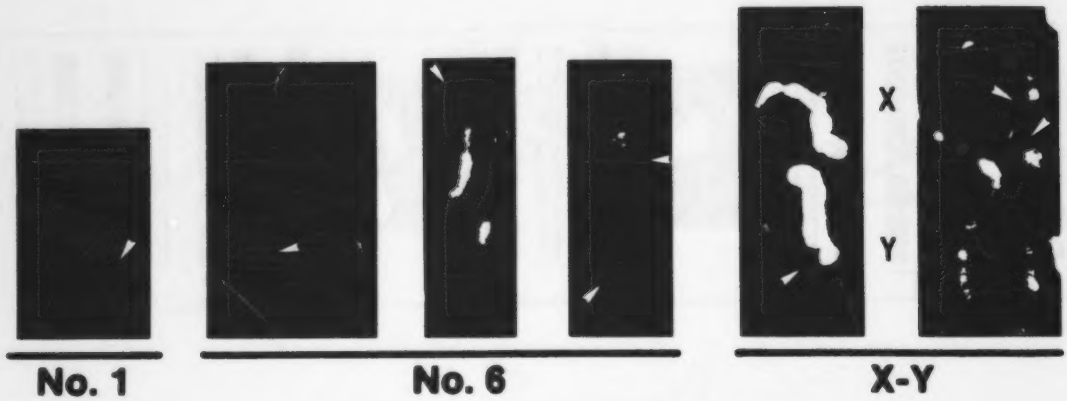


Figure 3

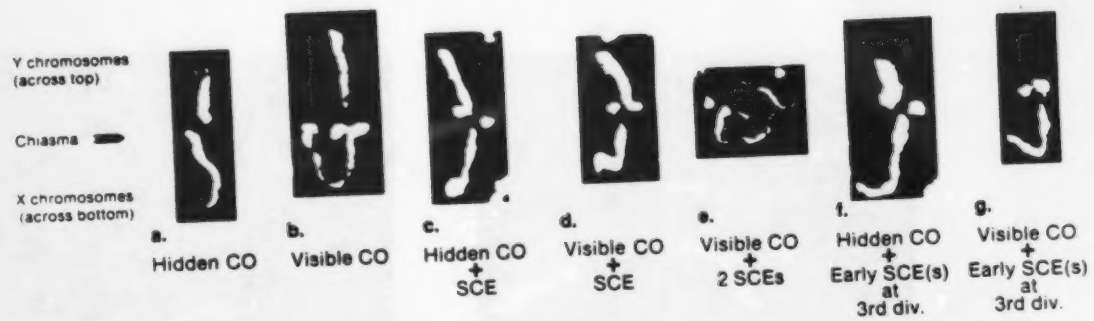
Y chromosomes (across top)
Chiasma
X chromosomes (across bottom)
a. Hidden CO
b. Visible CO
c. Hidden CO + SCE
d. Visible CO + SCE
e. Visible CO + 2 SCEs
f. Hidden CO + Early SCE(s) at 3rd div.
g. Visible CO + Early SCE(s) at 3rd div.

figure 4

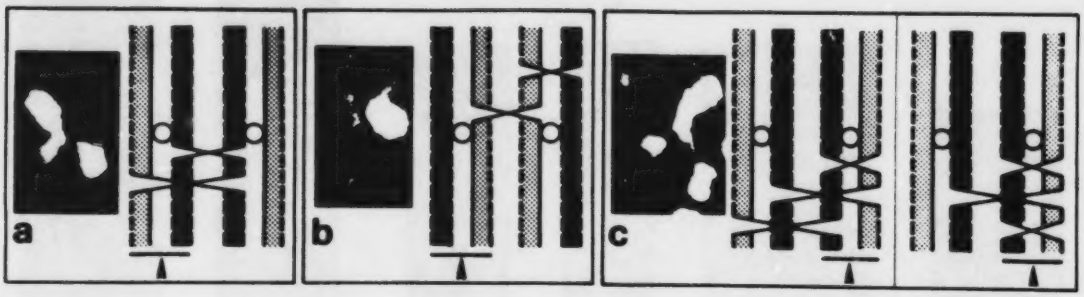


figure 5

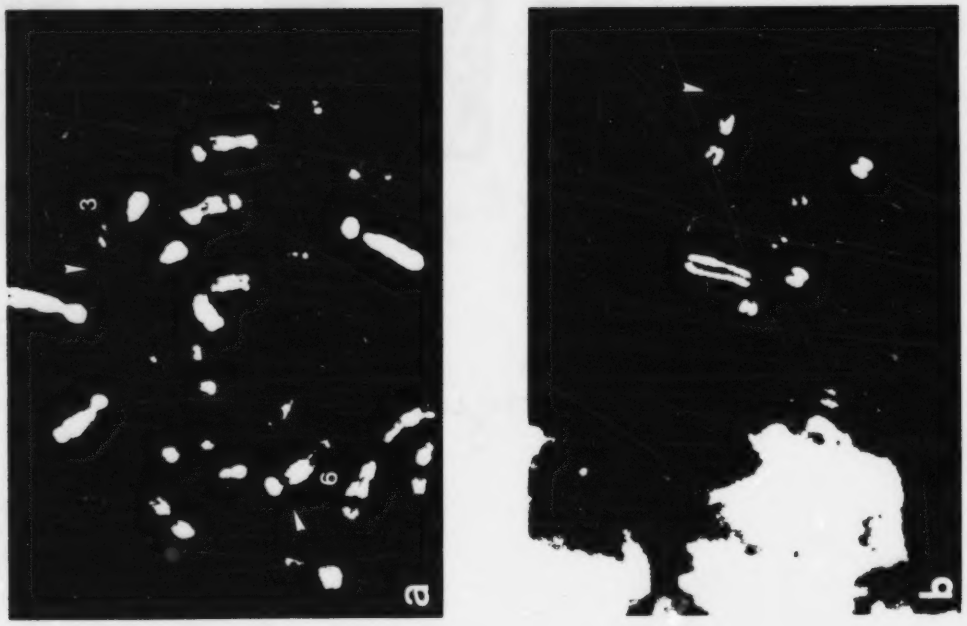


figure 6